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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

Date: March 19, 2008
TX Number: 0053833

SUBJECT: TETRACLORVINPHOS – Review of Developmental Neurotoxicity Study in Rats (MRID 46660601)

PC Code: 083701
DP Number: D323237

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THROUGH: Michael Metzger, Branch Chief
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I. CONCLUSIONS

The registrants, Hartz Mountain Corporation and KMG-Bernuth, Inc., submitted a developmental neurotoxicity study with TCVP in rats (MRID 46660601). The study was reviewed by the contractor, Dynamac Corporation, and went through the secondary review process in HED. This study was classified as acceptable/non-guideline. The DER for this study is attached to this memorandum and the citation and the conclusion of this study is presented below.

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II. & III. ACTION REQUESTED and BACKGROUND

The registrants, Hartz Mountain Corporation and KMG-Bernuth, Inc., submitted this study. SRRD requested RRB1, HED to review and prepare DER for this study.

IV. REVIEW SUMMARY

CITATION: Barnet, J.F. (2005) Oral (gavage) developmental neurotoxicity study of Tetrachlorvinphos in Crl:CD® (SD)IGS BR VAF/Plus® rats. CR-DDS Argus Division, Horsham, PA. Laboratory Project ID: CR-DDS Argus Protocol Number: 1608-003, September 9, 2005. MRID 46660601. Unpublished

In the developmental neurotoxicity study in rats, the pregnant dams were dosed (via gavage) from gestation day 6 to lactation day 6 at doses of 0, 10, 50, or 200 mg/kg/day. The pups were dosed at similar levels from lactation days 7-21. In dams, there was no evidence of maternal toxicity at any dose. In offspring, no treatment related effects were noted in the 10 or 50 mg/kg/day group. At 200 mg/kg/day (LOAEL), offspring toxicity was characterized by decreases in body weight and body weight gains during pre-weaning and post-weaning in both sexes, absolute brain weight in the males on PND 70, and several morphometric linear brain measurements in both sexes on PNDs 21 and 70. Morphometric brain measurements included thickness of the striatum, corpus callosum, hippocampal gyrus, and height of the cerebellum. The maternal NOAEL was 200 mg/kg/day (HDT). The offspring NOAEL was 50 mg/kg/day. In this study, cholinesterase (ChE) levels were not measured.

DATA EVALUATION RECORD

TETRACLORVINPHOS

Study Type (§83-6): Developmental Neurotoxicity Study in the Rat

Work Assignment No. 3-01-101 (MRID 46660601)

Prepared for

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Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

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Template version 11/01

DATA EVALUATION RECORD

STUDY TYPE: Developmental Neurotoxicity Study - Rat; OPPTS 870.6300 (§83-6); OECD 426 (draft)

PC CODE: 083701 **DP BARCODE:** D323237
TXR#: 0053833

TEST MATERIAL (PURITY): Tetrachlorvinphos (99.6% a.i.)

SYNONYMS: 2-Chloro-1-(2,4,5-trichlorophenyl)vinyl dimethyl phosphate

CITATION: Barnet, J.F. (2005) Oral (gavage) developmental neurotoxicity study of Tetrachlorvinphos in Crl:CD® (SD)IGS BR VAF/Plus® rats. CR-DDS Argus Division, Horsham, PA. Laboratory Project ID: CR-DDS Argus Protocol Number: 1608-003, September 9, 2005. MRID 46660601. Unpublished.

SPONSOR: Hartz Mountain Corporation, 400 Plaza Drive, Secaucus, NJ and KMG-Bernuth, Inc., 10611 Harwin Drive Ste. 420, Houston, TX

EXECUTIVE SUMMARY - In a developmental neurotoxicity study (MRID 46660601), Tetrachlorvinphos (99.6% a.i.; Lot #: NJ250RB08) in aqueous 1% (w/v) methylcellulose was administered via gavage (10 mL/kg) to pregnant Sprague-Dawley rats (25/dose) from gestation day (GD) 6 to lactation day (LD) 6 at doses of 0, 10, 50, or 200 mg/kg/day. Additionally, the F_1 pups were similarly dosed on postnatal days (PNDs) 7-21. Dams were allowed to deliver naturally and were sacrificed on LD 21. On PND 4, litters were standardized to 10 pups/litter; the remaining offspring and dams were sacrificed and subjected to a gross necropsy. Subsequently, 1 pup/sex/litter/group (at least 10 pups/sex/dose when available) were allocated to the following subsets: Subset 1, PND 21 brain weights and neurohistological evaluations; Subset 2, water maze and passive avoidance test; Subset 3, motor activity and auditory startle habituation; Subset 4, terminal brain weights and neurohistological evaluations; and Subset 5, standardize litter size to ten pups (5 male and 5 female) per litter on PND 4-21.

In dams, there were no treatment-related effects on mortality, clinical signs, body weight, body weight gain, feed consumption, FOB, or gross pathology. No treatment-related effects on reproductive parameters were observed. **The maternal LOAEL was not observed. The maternal NOAEL was 200 mg/kg/day (HDT).**

In offspring, there were no treatment-related effects on viability, litter observations, clinical signs, body weight, food consumption, FOB, motor activity, acoustic startle habituation, learning and memory (passive avoidance and water maze), gross pathology, or histopathology parameters. At 200 mg/kg/day, decreases ($p \leq 0.05$) in body weight were noted in the males (↓5-8%) on PNDs 15 and 16 (pre-weaning) and PND 22 (post-weaning) and in the females (↓6%) on PND 29 (post-weaning). Body weight gains were decreased ($p \leq 0.05$) during pre-weaning at several intervals in the males (↓12-28%) and females (↓13-26%). Decreases ($p \leq 0.05$) in body weight gains during post-weaning were observed in the males on PNDs 21-22 (↓36%) and in the females on PNDs 21-22 and 22-29 (↓7-28%). Absolute brain weight was decreased ($p \leq 0.01$) by 8% in the males. This finding was judged to correlate with the treatment-related decreases ($p \leq 0.05$) in several microscopic linear brain measurements: (i) thickness of the striatum on PND 21 (↓4-5% both sexes) and PND 70 (↓7% males only); (ii) thickness of the corpus callosum on PND 70 (↓16-21% both sexes); (iii) thickness of the hippocampal gyrus on PND 70 (↓7-9% both sexes); and (iv) height of the cerebellum on PND 70 (↓7% males only). It is noted that on PND 21, decrease in single morphometric parameter was seen in both sexes with the small magnitude (4-5%) whereas on PND 70, decrease in multiple morphometric measurements were seen in both sexes with greater magnitude (7-21%) than that seen in PND 21.

No treatment related effects were noted in the ≤50 mg/kg/day F_1 offspring.

The offspring LOAEL was 200 mg/kg/day, based on decreases in body weight, body weight gains and several morphometric linear brain measurements in both sexes, and decreased absolute brain weight in the males on PND 70. The offspring NOAEL was 50 mg/kg/day.

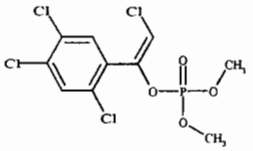
This study is classified **as Acceptable/Non Guideline** and may be used for regulatory purposes, however it does not satisfy the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, §83-6); OECD 426 at this time pending a comprehensive review of all available positive control data.

COMPLIANCE - Signed and dated Data Confidentiality, GLP Compliance, Flagging, and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material - Tetrachlorvinphos

Description:	White powder
Batch/Lot #:	NJ250RB08
Purity:	99.6% a.i.
Stability:	Not reported
CAS # of TGAI:	961-11-5
Structure:	

2. Vehicle - Aqueous 1% (w/v) methylcellulose

3. Test animals (P)

Species:	Rat
Strain:	Sprague-Dawley Crl:CD® (SD)IGS BR VAF/Plus®
Age at study initiation:	Approximately 10 weeks
Weight on GD 0:	226-256 g (females)
Source:	Charles River Laboratories, Inc. (Raleigh, NC)
Housing:	Dams were kept individually in stainless steel, wire-bottomed cages, except during cohabitation and postpartum periods. During mating, one male and one female were housed in the male's home cage. Beginning no later that GD 20, P females were individually housed in nesting boxes. The dams and their litters were housed in a common nesting box throughout the postpartum period. The F_1 animals were kept individually in stainless steel, wire-bottomed cages beginning on PND 20.
Diet:	Certified Rodent Diet® #5002 (PMI® Nutrition International, St. Louis, MO), *ad libitum*, except during behavioral testing.
Water:	Reverse osmosis treated tap water, with chlorine added as a bacteriostat, *ad libitum*, except during behavioral testing.
Environmental conditions:	
Temperature:	18-26 °C
Humidity:	30-70%
Air changes:	≥10/hr
Photoperiod:	12 hrs light/12 hrs dark
Acclimation period:	5 days

B. PROCEDURES AND STUDY DESIGN

1. In-life dates - Start: 03/07/04 End: 06/11/04

2. Study schedule - The maternal animals were mated and assigned to study. The F_0 females were administered the test substance daily via gavage (10 mL/kg) from gestation day (GD) 6 until lactation day (LD) 6 or GD 25 (rats that did not deliver a litter). The F_1 pups were administered the test substance daily via gavage (10 mL/kg) from postnatal day (PND) 7 until PND 21 (weaning). On PND 4, the litters containing >10 pups were randomly standardized to

10 pups/litter (with equal sexes where possible) to reduce the variability. All other litters and all P females without a litter were sacrificed and discarded without further examinations. F_1 pups remained on study for up to PND 71 (study termination).

3. **Mating procedure** - The animals were mated at the test facility by placing one male and one female in the male's cage for a period of up to 4 days. Successful mating was verified by the presence of sperm in a vaginal smear and/or a copulatory plug. The day of successful mating was designated GD 0.

4. **Animal assignment** - Pregnant females were randomly assigned (based on body weight) to test groups as shown in Table 1. Dams were assigned to functional observation testing as shown. Offspring were assigned to testing subsets at the time of litter standardization on PND 4.

Table 1. Study design [a]

Experimental Parameter	Subset	Dose (mg/kg/day)			
		0	10	50	200
Dams					
# of maternal animals	NA	25	25	25	25
FOB (GD 6 through LD 6 or 25[b])	NA	25	25	25	25
Offspring					
FOB (PND 4, 11, 21, 35, 45, 60)	4	All pups	All pups	All pups	All pups
Motor activity (PND 13, 17, 21, and 60 or 61)	3	1 pup/sex/litter	1 pup/sex/litter	1 pup/sex/litter	1 pup/sex/litter
Auditory startle habituation (PND 22, 60)	3	1 pup/sex/litter	1 pup/sex/litter	1 pup/sex/litter	1 pup/sex/litter
Learning and Memory Passive avoidance (PND 22-24 and 29-31) Water maze (PND 58-62 and 65-69)	2	1 pup/sex/litter 1 pup/sex/litter	1 pup/sex/litter 1 pup/sex/litter	1 pup/sex/litter 1 pup/sex/litter	1 pup/sex/litter 1 pup/sex/litter
Brain weight and neuropathology (including morphometry) [c] (PND 21, 22 or 23) (PND 70)	 1 4	 10 pups/sex 10 pups/sex	 10 pups/sex 10 pups/sex	 10 pups/sex 10 pups/sex	 10 pups/sex 10 pups/sex
Animals to standardize litter size	5	17 M/15 F	20 M/13 F	16 M/12 F	13 M/12 F

a Data were obtained from pages 32-43 of the study report.
b Rats that did not deliver a litter.
c At each sacrifice time 1 pup/litter was taken to give at least 10 pups/sex/dose.

5. Dose selection rationale - The doses presented in Table 1 were selected by the Sponsor based on the results of cholinesterase evaluation studies (Argus Study Nos. 1608-001 and 1608-002R cited in MRID 46036001).

6. Dosage preparation, administration, and analysis - Test formulations were prepared daily by mixing the appropriate amount of the test material with aqueous 1% (w/v) methylcellulose. The test formulations were stored at room temperature until use. The F_0 females were administered the test substance daily via gavage (10 mL/kg) from GD 6 until LD 6 or GD 25 (rats that did not deliver a litter). The F_1 pups were administered the test substance daily via gavage (10 mL/kg) from PND 7 until PND 21 (weaning). Homogeneity was not determined; however, it was stated that the dose formulations were stirred continuously using a magnetic stirrer during preparation, sampling, aliquotting, and dosing. Stability was not reported; however, it was stated that stability data for prepared formulations bracketing those in the current study were provided by the Sponsor to the Testing Facility. Concentration was determined from samples of all doses at the beginning and end of the treatment period.

Results

Concentration (range as % of nominal):

Dose (mg/kg/day)	% of Nominal
10	84.6-100.8
50	89.8-90.9
200	76.3-109.6

At 200 mg/kg/day, the concentration range (% of nominal) was highly variable. Three of the four samples were below the acceptable ±15% range (start of study: 76.3% and 80.6%; end of study: 81.3%). The remaining 200 mg/kg/day sample was 109.6% of nominal. Without more samples at interim time points, it cannot be determined if there was a problem with the analytical method or with the preparation method. It was stated that the viscosity of the dose preparations did increase with increasing dose and the dose formulations were stirred continuously using a magnetic stirrer during preparation, sampling, aliquotting, and dosing. The analytical data indicated the mixing procedure was marginally adequate and the variation between nominal and actual dosage to the study animals was acceptable.

C. OBSERVATIONS

1. In-life observations

a. Maternal animals - The dams were checked at least twice daily for mortality. Clinical signs of toxicity and general appearance were evaluated weekly during the acclimation period and on GD 0, and daily during the post-treatment period. Body weights were measured weekly during the acclimation period, on GD 0, daily during the treatment period, on LDs 7, 11, 14, 17, and 21, and at termination. Feed consumption was recorded on GD 0, and daily during the treatment and

post-treatment periods. Feed consumption was not tabulated after LD 13, when it was expected that pups would start consuming the maternal feed. The dams were also evaluated for adverse clinical signs observed during parturition, duration of gestation, litter size, live litter size, and pup viability at birth. Maternal behavior was evaluated on LDs 0, 4, 7, 13, and 21.

The dams were subjected to a modified functional observation battery (FOB) outside of the home cage daily, prior to dosing, beginning on GD 6 and continuing through LD 6 or GD 25 (rats that did not litter). The technicians were 'blind' as to the animal's dose group. The functional observations included, but were not limited to the following.

	FUNCTIONAL OBSERVATIONS
X	Signs of autonomic function, including: 1) Lacrimation and salivation 2) Piloerection 3) Respiration 4) Palpebral closure 5) Exophthalmos 6) Urination and defecation
X	Description, incidence, and severity of any convulsions, tremors, or abnormal movements.
X	Description and incidence of posture and gait abnormalities.
X	Description and incidence of any unusual or abnormal behaviors, excessive or repetitive actions, and general signs of toxicity (thin, altered muscle tone, dehydrated, or altered fur appearance).

b. Offspring

1) Litter observations - Pups were evaluated for mortality and morbidity at least twice daily throughout the study and were counted once daily. Clinical observations were recorded daily during pre-treatment (PNDs 0-6), daily prior to dosing during the treatment period (PNDs 7-21), and weekly during the post-treatment period (PND 22 to termination). Observations for clinical signs during the treatment period were performed at 60 to 120 minutes post-dosing. Body weights were recorded on PNDs 0 and 4 (precull and post-cull); daily during PNDs 7-21; and then weekly thereafter until sacrifice. Post-weaning food consumption was recorded weekly.

On PND 4, the litters containing >10 pups were standardized to 10 pups/litter (with equal sexes where possible) to reduce the variability. Litters not selected for F_1 evaluations and excess pups were sacrificed and necropsied.

2) Developmental landmarks - Beginning on PND 38, selected male offspring (Subsets 2-4) were examined daily for preputial separation. Beginning on PND 27, selected female offspring (Subsets 2-4) were examined daily for vaginal patency. The body weights were recorded for each animal on the day sexual maturation was confirmed.

3) Postweaning observations - After weaning (PND 21), offspring were examined for mortality and morbidity at least twice daily. Clinical observations were made weekly during post-weaning and animals were examined for gross signs of toxicity after each weighing. Body weights were recorded for each animal on the day of preputial separation or vaginal patency.

4) Neurobehavioral evaluations

i) Functional observational battery (FOB) - On PNDs 4, 11, 21, 35, 45, and 60 all animals were subjected to a modified FOB in the open-field, as appropriate for the developmental stage being observed. The same parameters assessed in the maternal FOB were examined in the offspring. The technicians were 'blind' as to the dose group; however, it was not reported if the same technicians performed the evaluations at each time point. The scoring criteria for the FOB were not provided.

ii) Motor activity testing - Motor activity measurements (number of movements and time spent in movement) were performed on selected animals (Subset 3; 1 pup/sex/litter) on PNDs 13, 17, and 21 pre-dosing and PND 60 or 61. Movement was monitored using a passive infrared sensor mounted outside a stainless steel, wire-bottomed cage (Plexiglass® flooring was used for pups during pre-weaning). Data were collected in 10-minute intervals over the course of 1 hour. Groups were counter balanced across testing sessions and cages.

iii) Auditory startle reflex habituation - Auditory startle response and habituation of responses with repeated presentation of stimuli were evaluated for selected animals (Subset 3; 1 pup/sex/litter/dose group) on PNDs 22 and 60. The rats were tested in sets of 4 within a sound attenuated chamber. Each animal was placed in a small cage on a platform containing a force transducer in its base. A computer sampled the transducer output and controlled the test session. The animals initially underwent an adaption period of 5 minutes. In the last minute of the adaptation period, 10 'blank' trials were conducted to sample the baseline force in the absence of a stimulus. The animals were then presented with 120 dB bursts of 30 msec duration at 10-second interval for 50 trials, followed by another 10 'blank' trials. The peak amplitude of each response was recorded, and the mean baseline response amplitude was subtracted to calculate the response magnitude. The mean response magnitude and the pattern of responses over 10 trial blocks were compared among dose groups.

iv) Learning and memory testing - Learning and memory testing was performed on the same group of rats (Subset 2; 1 pup/sex/litter/ dose group) at two different occasions. Passive avoidance testing was performed on PNDs 22 to 24 and again one week later, and water maze testing was performed on PNDs 58 to 62 and again one week later.

Passive avoidance test - Individual animals were placed into the 'bright' side of a two-compartment chamber. The trial began with the light being illuminated to signal the beginning of the trial and the door separating the two compartments opening, so that the animal was provided access to the non-illuminated side of the cage. Once the rat crossed into the 'dark' compartment, the door was closed, a shock (1 mA for 1 sec) was delivered to the grid floor, and the light was switched off, signaling the end of the trial. At that time the animal was returned to the holding cage for 30 seconds to await the next trial. Trials were repeated until either the rat remained in the lighted compartment for 60 seconds on two consecutive trials or until 15 trials had elapsed, whichever occurred first. The test was repeated one week later using the same test conditions and criteria. The following dependent measures were compared among the dose groups: (i) number of trials to criterion in the first session (overall learning performance); (ii)

latency (in sec) to enter the dark compartment in Trial 1 of the first session (activity level); (iii) latency (in sec) to enter the dark compartment in Trial 2 of the first session (short-term retention); (iv) number of trials to criterion in the second session (long-term retention); and (v) latency (in sec) to enter the dark compartment in Trial 1 of the second session (long-term retention).

Water maze - A modified M-maze constructed of stainless steel was used. The water level was approximately 9 inches and the water temperature was 21±1 °C. On each test trial, the rat was placed into the starting position at the base of the M-maze stem, located between the two lateral arms. On the first trial (learning trial), the rat was required to enter both arms of the maze before being provided access to the exit ramp to escape the water and then removed from the maze. The initial arm chosen on this leaning trial was designated the incorrect goal during the subsequent trials (15 maximum). Rats that failed to make a correct goal choice within 60 seconds in any given trial were guided to the correct goal with the exit ramp and then removed from the water. Between trials, the animal was returned to a holding cage for 15 seconds to wait for the next trial. Each rat was required to reach a criterion of five consecutive errorless trials to terminate the test session. The maximum number of trials in any test session was 15. Latency to choose the correct goal or the maximum 60-second interval was recorded for each trial, as was the number of errors during each trial. All animals were tested again one week later using the same test conditions and criteria. The following dependent measures were compared among the dose groups: (i) number of trials to criterion in the first session (overall learning performance); (ii) mean number of errors (incorrect turns in the maze) for each trial during the first session (overall learning performance); (iii) latency (in sec) to reach the correct goal in Trial 2 of the first session (short-term retention); (iv) number of trials to criterion in the second session (long-term retention); (v) mean number of errors (incorrect turns in the maze) for each trial during the second session (long-term retention); and (vi) latency (in sec) to reach the correct goal in Trial 2 of the second session (long-term retention).

2. Postmortem observations - All sacrifices were performed via CO_2 asphyxiation and a gross necropsy of the thoracic, abdominal, and pelvic viscera was performed. All gross lesions were retained in neutral buffered 10% formalin and all other tissues were discarded. The number and distribution of implantation sites were recorded in all dams.

a. Maternal animals - Dams that delivered a litter and were selected for continued observation on LD 4 were sacrificed on LD 21. Dams that delivered a litter but were not selected for continued observation were sacrificed on LD 4. Dams that delivered a litter but did not have sufficient pups for subset assignment were sacrificed on LDs 4 and 6. Rats not delivering a litter were sacrificed on GD 25 and the uteri were examined to verify their non-pregnant condition.

b. Offspring - Pups that died before initial examination of the litter were evaluated for vital status at birth by immersing the lungs in water. Lungs that floated were considered live-born and those that sank were considered stillborn. All pups found dead were examined for gross lesions

and the cause of death on the day that the observation was made. Pups culled on PND 4 were sacrificed and necropsied. Pups with gross lesions were preserved in Bouin's solution.

The animals selected for sacrifice on PND 21, 22, or 23 (Subset 1; 10/sex/dose) were administered a combination of sodium heparin (0.2 mg/mL) and sodium pentobarbital (0.06 mg/mL), and were perfused *in situ* with neutral buffered 10% formalin. The animals were examined for gross lesions, and the brains, heads, spinal columns and hindlimbs were shipped (in neutral buffered 10% formalin) for weighing, morphological measurements, and neurohistological evaluations. Additionally, animals in Subset 5 were sacrificed and necropsied on these days.

On PNDs 60 or 61, selected animals in Subset 4 (10/sex/dose) were sacrificed, perfused *in situ*, and routinely processed for neuropathological evaluation as described above for the Subset 1 animals. All Subset 4 animals not selected for these procedures and all other surviving animals were sacrificed via CO_2 asphyxiation and necropsied.

The CHECKED (X) tissues listed below were removed from all animals and preserved in neutral buffered 10% formalin.

	CENTRAL NERVOUS SYSTEM		PERIPHERAL NERVOUS SYSTEM
	BRAIN		**SCIATIC NERVE**
X	Olfactory bulbs	X	Sciatic nerve
X	Frontal lobe		
X	Parietal lobe		**OTHER**
X	Midbrain with occipital and temporal lobe	X	Sural nerve
X	Pons	X	Tibial nerve
X	Medulla oblongata	X	Peroneal nerve
X	Cerebellum	X	Lumbar dorsal root ganglion
	SPINAL CORD	X	Lumbar dorsal root fibers
X	Cervical swelling	X	Lumbar ventral root fibers
X	Lumbar swelling	X	Cervical dorsal root ganglion
	OTHER	X	Cervical dorsal root fibers
X	Gasserian ganglia with trigeminal nerve	X	Cervical ventral root fibers
	Pituitary gland		
	Eyes (with retina and optic nerve)		
	Skeletal muscle (gastrocnemius)		

The brain, spinal cord, Gasserian ganglion, nerve roots, and dorsal root ganglia were embedded in paraffin, sectioned (5 μm), and stained with hematoxylin and eosin and luxol fast blue/cresyl

violet (Subsets 1 and 4) and Bielschowsky's technique (Subset 4). The peripheral nerves (sciatic, tibial, sural, and peroneal) were embedded in glycol methacrylate, sectioned (2 µm), and stained with hematoxylin and eosin, toluidine blue, and Bielschowsky's technique. The brain sections (Subsets 1 and 4) and the other tissues (Subset 4) from the control and 200 mg/kg/day animals were examined microscopically. The following linear morphometric measurements were performed: thickness of the frontal cortex; thickness of the parietal cortex; diagonal width of the striatum (caudate plus putamen); thickness of the corpus callosum; thickness of the hippocampal gyrus; and maximum height of the cerebellum. Additionally, the length of the cerebrum (anterior to posterior, excluding the olfactory bulbs) and the cerebellum (anterior edge of the cerebellar cortex to posterior pole) were obtained prior to brain sectioning.

D. DATA ANALYSIS

1. Statistical analyses - In general, continuous data (body weight, food consumption, latency and errors per trial scores in behavioral tests, and percent mortality per litter) were initially assessed for equality of variance using Bartlett's test. Group means with equal variances were analyzed further using ANOVA, followed by Dunnett's test as necessary. Group means with unequal variances were analyzed using non-parametric procedures; either a Kruskal-Wallis test followed by Dunn's test (if ≤75% of the scores were tied) or Fisher's Exact test (if >75% of the scores were tied).

Motor activity and auditory startle habituation interval data were analyzed using a repeated measures ANOVA for a Dosage effect or a Dosage x Block interaction. If the Dosage effect was significant the data were analyzed using Dunnett's test. If the Dosage x Block interaction was significant, the data at each measurement point were analyzed further using ANOVA, followed by Dunnett's test as necessary.

Litter size, number of trials to criterion, and developmental landmark data were analyzed using non-parametric procedures; either a Kruskal-Wallis test followed by Dunn's test (if ≤75% of the scores were tied) or Fisher's Exact test (if >75% of the scores were tied).

Clinical observations and other proportion data were analyzed as contingency tables using Variance Test for Homogeneity of the Binomial Distribution. Gross linear brain measurements were analyzed using ANOVA, followed by Dunnett's test as necessary. The level of significance was set at $p \leq 0.05$ for all tests (except Bartlett's, $p \leq 0.001$).

2. Indices - The following indices were calculated by the Sponsor:

Gestation index = # rats with live offspring/# pregnant rats

Viability index = # live pups on PND 4 (pre-cull)/# liveborn pups on PND 0

3. Positive control data - Positive control data that validate the procedures and observers of the performing lab to assess motor activity, neurotoxicity and behavioral effects were not provided with the current study. However, the following summary positive control data, already on file with the Agency, were available (MRID 46179301, Argus Research Laboratories). Acrylamide (30 mg/kg in saline, i.p. for 17 days) caused significant differences in gait, limb splay, movement, number of rears in the open field, unusual posture, appearance, tail pinch, air righting, grip strength and rectal temperature. Trimethyltin (8 mg/kg in saline, i.p. once) caused significant differences in reaction to removal and auditory reaction 7 days post-dosing. MK-801 (0.3 mg/kg in corn oil, i.p. once) provided significant differences in home cage behavior, stereotypic behavior, gait alterations, tactile reactivity, air righting response, hindlimb grip strength and landing foot splay within 30 minutes post-dosing. Carbaryl (100 mg/kg in corn oil, once via oral gavage) provided significant differences in home cage behavior, posture, whole body tremors or spasms, gait alterations, increased salivation, tactile and auditory reactivity, air righting response, landing foot splay and rectal temperatures within 30 minutes post-dosing. DDT (100 mg/kg in corn oil, once via oral gavage) provided significant differences in home cage behavior, whole body tremors or spasms, gait alterations, and rectal temperatures at 6 hours post-dosing. Analysis of the interobserver variability was performed on selected FOB parameters for each test substance. There were no statistically significant differences among the observers.

II. RESULTS

A. PARENTAL ANIMALS

1. Mortality, clinical signs, and functional observations - All dams survived to scheduled sacrifice. No treatment-related clinical signs or FOB effects were noted at any dose throughout the study.

2. Body weight and feed consumption - Selected group mean body weights and feed consumption values for pregnant or nursing dams are summarized in Tables 2 and 3, respectively. Body weights and body weight gains for the P females were similar to controls throughout the gestation and lactation periods.

Table 2. Selected mean (± SD) body weights (g) for P females administered Tetrachlorvinphos daily via gavage from GD 6 to LD 6. [a]

Interval (Days)	Dose (mg/kg/day)			
	0	10	50	200
Gestation (n=23-25)				
0	241.3±8.5	241.7±8.3	241.0±8.4	242.1±9.3
6	275.4±13.4	274.0±13.0	272.7±11.6	273.2±11.9
20	389.3±24.8	396.3±23.8	387.5±28.4	382.4±23.4
6-20	113.9±17.2	122.2±15.8	114.8±21.5	109.2±15.6
0-20	148.0±20.7	154.6±19.4	146.5±26.0	140.4±19.2
Lactation (n=20-25)				
0	295.2±19.1	299.1±21.1	294.5±20.8	289.9±15.8
5[b]	307.2±25.2	308.1±24.2	306.0±22.1	305.4±19.0
21[b]	329.6±25.1	337.5±19.2	326.2±19.6	338.7±19.8
0-7	17.9±14.8	19.0±11.3	20.8±16.1	24.8±11.7
7-11	24.2±17.7	16.6±9.3	15.3±9.6	17.6±11.8
7-21	18.8±22.2	18.8±14.8	11.6±16.6	23.1±18.6
0-21[c]	34.4	38.4	31.7	48.8

a Data were obtained from Tables B2-B5, pages 107-110 of the study report.
b Excludes values for dams that were not selected for continued observation or sacrificed due to no surviving pups.
c Calculated by the reviewers from the data contained within this table.

During gestation, absolute (g/animal/day) and relative (g/kg/day) feed consumption were similar to controls at all doses. During lactation, relative feed consumption was reduced ($p \leq 0.05$) by 8% in the 200 mg/kg/day dams during LD 7-11 (Table 3). However, as this decrease was minor and there was no significant decreases in body weight or body weight gain during this interval, this finding was not considered to be toxicologically important. The increased relative feed consumption noted in the 200 mg/kg/day dams at LD 0-3 (↑13%, $p \leq 0.05$) was not considered to be adverse. Absolute feed consumption was similar to controls at all doses during the lactation period.

Table 3. Selected mean (± SD) absolute (g/animal/day) and relative (g/kg/day) food consumption for P females administered Tetrachlorvinphos daily via gavage from GD 6 to LD 6.[a]

Interval (Days)		Dose (mg/kg/day)			
		0	10	50	200
Gestation (n=22-25)					
Absolute	0-6	26.1±2.8[b]	26.2±2.8	26.0±2.5	26.4±4.0
	6-20	25.8±2.6	26.4±2.7	25.8±2.9	25.4±2.4
	Overall (0-20)	25.9±2.5	26.4±2.6	25.8±2.5	25.7±2.6
Relative	0-6	100.8±7.4[b]	101.5±8.1	101.3±8.9	102.2±13.9
	6-20	81.0±4.8	82.6±5.6	81.7±6.0	81.6±4.5
	Overall (0-20)	82.4±4.4	83.7±5.2	83.2±5.4	83.6±5.6
Lactation (n=18-25)					
Absolute	0-3	30.7±6.2	31.1±5.5	30.9±5.5	34.0±6.2
	0-7[c]	39.1±6.2	39.3±4.8	40.2±5.5[d]	42.2±4.6[d]
	7-13[c]	62.2±6.8	60.3±7.4	61.6±7.2	57.6±7.6
Relative	0-3	102.1±16.7	102.9±15.8	104.2±17.6	115.8±20.6* (↑13)
	0-7[c]	128.9±14.9	128.0±13.0	133.0±15.7[d]	139.8±13.9[d]
	7-11[be]	189.8±15.4	183.4±15.2	190.4±18.4	174.7±22.1* (↓8)

a Data were obtained from Tables B6-B9, pages 111-114 of the study report. Percent difference from controls (calculated by reviewers) is listed parenthetically.
b Excludes values that were associated with spillage.
c Excludes values for dams not selected for continued observation.
d Excludes values that appeared incorrectly recorded.
e Because it was presumed that the pups began to consume maternal feed after LD 13, maternal feed consumption values were not tabulated on LDs 13-21.
* Statistically significantly different from controls at $p \leq 0.05$

3. **Reproductive performance** - Pregnancy rate, gestation index, number of implantations/litter, and gestation length were comparable between treated and control animals (Table 4).

Table 4. Delivery observations in P females administered Tetrachlorvinphos daily via gavage from GD 6 to LD 6. [a]

Observation	Dose (mg/kg/day)			
	0	10	50	200
# Animals Mated	25	25	25	25
# Animals Pregnant Pregnancy Rate (%)[b]	23 92	25 100	25 100	25 100
# Nonpregnant	2	0	0	0
Gestation Index (%)[c]	100	100	100	100
Mean (±SD) gestation length (days)	22.5±0.5	22.7±0.5	22.5±0.5	22.4±0.5
Total # Implantations Mean (±SD) Implantations/Delivered Litter	353[d] 16.0±2.1[d]	403 16.1±1.7	403 16.1±2.1	390 15.6±1.8
Total # of Litters Examined	23	25	25	25

a Data were obtained from Table B10, page 115 of the study report.
b Calculated by the reviewers using data within this table.
c Number of rats with live offspring/number of pregnant rats.
d Excludes values for dam 7233; number of implantation sites appeared incorrectly recorded.

4. Maternal postmortem results

a. Macroscopic examination - No treatment-related gross lesions were observed at any dose.

b. Microscopic examination - Microscopic examinations were not conducted on the dams.

B. OFFSPRING

1. Viability and clinical signs - No treatment-related differences in live litter size, post-natal survival, viability index, or sex ratio were observed in any treated group (Table 5). No treatment-related clinical signs were observed at any dose. Mortality was increased in the 200 mg/kg/day males (15/101 treated vs. 0/100 controls, $p \leq 0.01$, Table 6). However, during the necropsy it was determined that 8/15 deaths were the result of injuries caused during dosing. Therefore, this finding was considered unrelated to treatment.

Table 5. F_1 live litter size and viability. [a]

Observation	Dose (mg/kg/day)			
	0	10	50	200
Number of litters	23	25	25	25
Total Number of Pups Born	342	378	374	361
Number Liveborn (%)	341 (99.7)	376 (99.5)	373 (99.7)	361 (100)
Number Stillborn	1	2	1	0
Sex Ratio PND 0 (% male)	53.0±13.0	47.8±12.4	52.1±12.7	54.4±11.8
# of Deaths PND 0 (%)	1 (0.3)	1 (0.3)	0 (0)	2 (0.6)
# of Deaths PNDs 1-4 (%)	5 (1.5)	7 (1.9)	6 (1.6)	10 (2.8)
Survival (pups/litter)				
PND 0	14.8±2.3	15.0±1.8	14.9±2.2	14.4±2.8
PND 4[b]	14.6±2.3	14.7±1.8	14.7±2.2	14.0±2.6
PND 4[c]	10.4±1.8	10.8±2.1	10.7±2.5	10.5±2.0
PND 7	NR	NR	NR	NR
PND 11	NR	NR	NR	NR
PND 17	NR	NR	NR	NR
PND 21	NR	NR	NR	NR
Viability index (%)[d]	98.2	97.9	98.4	96.7

a Data were obtained from Table B11, pages 116-117 of the study report.
b Before culling
c After culling
d # live pups on PND 4 (pre-cull)/# liveborn pups on PND 0
NR Not reported

Table 6. Mortality in F_1 pups administered Tetrachlorvinphos daily via gavage from PND 7-21. [a]

Observation	Dose (mg/kg/day)			
	0	10	50	200
Males (n=99-101)				
Total Deaths	0	1	4	15**[b]
Gavage Errors	0	1	3	8
Females (n=98-100)				
Total Deaths	4	7	11	13
Gavage Errors	3	3	6	3

a Data were obtained from pages 50-63 and Table C1and C2, pages 194 and 197 of the study report.
b Includes one moribund sacrifice.
** Statistically significantly different from controls at $p \leq 0.01$

2. **Body weight and feed consumption** - Selected pre- and post-weaning body weights and body weight gains for F_1 pups are presented in Tables 7a and 7b, respectively. At 200 mg/kg/day, decreases ($p \leq 0.05$) in body weight were noted in the males (↓5-8%) on PNDs 15 and 16 (pre-weaning) and PND 22 (post-weaning) and in the females (↓6%) on PND 29 (post-weaning). All other differences from controls were considered unrelated to treatment because they were minor, transient, and/or not-dose dependent.

Table 7a. Selected mean (± SD) body weights (g) in F_1 pups administered Tetrachlorvinphos daily via gavage from PND 7-21. [a]

Postnatal Day	Dose (mg/kg/day)			
	0	10	50	200
Males				
Pre-weaning (n=90-101)				
7	15.0±2.9	16.0±2.0	15.4±3.1	14.4±3.2
15[b]	33.0±5.6	33.9±4.6	33.7±6.2	31.0±6.9* (↓6)
16[b]	35.4±5.8	36.2±5.0	36.0±6.2	33.6±7.2* (↓5)
21[b]	48.6±7.7	49.0±7.7	49.1±7.8	46.7±9.2
Post-weaning (n=46-60)[c]				
22	53.9±8.3	54.0±8.4	52.4±9.2	49.4±10.6* (↓8)
71[d]	405.9±37.1	419.0±39.8	405.8±40.1	414.6±36.6
Females				
Pre-weaning (n=88-100)				
7	14.3±2.6	15.1±2.1* (↑6)	14.6±2.7	14.0±3.0
15[b]	31.8±5.2	32.0±4.9	32.5±5.7	30.8±5.1
21[b]	46.5±6.7	46.0±7.6	46.6±7.0	45.0±7.2
Post-weaning (n=49-60)[c]				
22	50.2±8.1	49.8±8.5	49.5±7.9	47.5±8.8
29[b]	87.1±12.1	87.6±12.3	84.5±10.6	82.2±11.9* (↓6)
71[d]	245.6±24.9	254.4±24.9	241.0±21.3	247.4±24.1

a Data were obtained from Tables C5 and C7, pages 203-204 and 207-208 of the study report. Percent difference from controls (calculated by reviewers) is listed parenthetically.
b Excluding values for animals found dead or moribund sacrificed
c Excluding animals from Subsets 1 and 5
d Excluding animals sacrificed prior to PND 71
* Statistically significantly different from controls at $p \leq 0.05$

At 200 mg/kg/day, body weight gains were decreased ($p \leq 0.05$) compared to controls in the males on PNDs 7-8, 8-9, 14-15, and 19-20 (↓12-28%, pre-weaning) and PNDs 21-22 (↓36%, post-weaning, Table 7b) and in the females on PNDs 7-8, 8-9, 11-12, 18-19, and 19-20 (↓13-26%, pre-weaning) and PNDs 21-22 and 22-29 (↓7-28%, post-weaning). At 50 mg/kg/day, body weight gains were decreased ($p \leq 0.01$) compared to controls in the males on PNDs 21-22 (↓23%, post-weaning). All other differences from controls were considered unrelated to treatment because they were minor, transient, and/or not dose-dependent.

Table 7b. Selected mean (±SD) body weight gains (g) in F_1 pups administered Tetrachlorvinphos daily via gavage from PND 7-21. [a]

Interval (PND)	Dose (mg/kg/day)			
	0	10	50	200
Males				
Pre-weaning (n=90-101)				
7-8	1.8±0.8	2.0±0.8	1.9±1.0	1.3±1.0** (↓28)
14-15[b]	2.5±0.6	2.4±0.8	2.4±0.8	2.2±0.9** (↓12)
19-20[b]	3.1±1.3	2.9±1.5	3.1±1.3	2.5±1.5** (↓19)
7-21[b]	33.6±5.3	33.0±6.4	33.6±5.5	32.0±6.5
Post-weaning (n=46-60)[bc]				
21-22	4.7±1.8	4.7±1.8	3.6±2.2** (↓23)	3.0±2.3** (↓36)
22-71[d]	352.6±31.7	364.9±36.2	353.8±33.9	364.5±32.5
Females				
Pre-weaning (n=88-100)				
7-8[b]	1.9±0.9	1.9±0.7	1.8±1.1	1.4±1.0** (↓26)
11-12[b]	2.3±1.0	2.3±0.7	2.4±0.8	2.0±0.8** (↓13)
19-20[b]	2.9±1.3	2.7±1.3	2.9±1.4	2.4±1.2* (↓17)
7-21[b]	32.2±4.8	30.9±6.2	31.9±5.3	30.6±5.2
Post-weaning (n=49-60)[bc]				
21-22	3.9±1.8	3.7±2.1	3.2±2.0	2.8±2.2** (↓28)
22-29[b]	36.9±5.4	37.8±5.0	35.0±4.2* (↓5)	34.2±5.1** (↓7)
22-71[d]	194.8±22.8	204.8±21.9* (↑5)	192.4±20.0	199.2±23.4

a Data were obtained from Tables C6 and C8, pages 205-206 and 209-210 of the study report. Percent difference from controls (calculated by reviewers) is listed parenthetically.
b Excluding values for animals found dead or moribund sacrificed
c Excluding animals from Subsets 1 and 5
d Excluding animals sacrificed prior to PND 71
* Statistically significantly different from controls at $p \leq 0.05$
** Statistically significantly different from controls at $p \leq 0.01$

Absolute (g/animal/day) and relative (g/kg/day) feed consumption were comparable between treated and control animals. The differences ($p \leq 0.05$) noted in the 10 and 200 mg/kg/day males and in the 10 mg/kg/day females were considered unrelated to treatment as they were minor, transient, and/or not dose-dependent.

3. Developmental landmarks

a. Sexual maturation - No treatment-related differences in balanopreputial separation or vaginal patency were observed between treated and control F_1 animals (Table 8).

Table 8. Sexual maturation (mean days ± SD) in F_1 generation rats administered Tetrachlorvinphos daily via gavage from PND 7-21. [a]

Parameter	Dose (mg/kg/day)			
	0	10	50	200
Number of Litters	20	20	20	20
Preputial Separation (Males)	45.3±2.0	45.3±2.4	46.0±2.0	46.5±2.1
Vaginal Patency (Females)	32.8±2.5	33.0±2.4	33.7±2.4	32.7±2.8

a Data were obtained from Table C13, page 215 of the study report.

b. Physical landmarks - Evaluation of physical landmarks was not performed.

4. Behavioral assessments

a. Functional observational battery - It was stated that no treatment-related FOB findings were observed; however, no FOB data were provided.

b. Motor activity - Mean total session motor activity (number of movements and time spent in movement) was similar to controls at all doses in both sexes at all time points (Table 9). Likewise, no significant differences were observed at any sub-session interval at any dose in either sex.

Table 9. Mean (±SD) number of movements (counts) and time spent in movement (sec) during motor activity assessment in F_1 pups in Subset 3 administered Tetrachlorvinphos daily via gavage from PND 7-21. [a]

Postnatal Day	Dose (mg/kg/day)			
	0	10	50	200
Males				
Number of Movements (counts)				
13	392.2±212.7	328.0±175.3	383.4±212.4	395.0±243.3
17	524.9±208.3	582.8±229.2	633.2±183.2	628.8±229.6
21	562.1±124.3	494.5±201.8	540.8±189.7	574.2±246.4
60	781.5±136.0	733.9±134.5	766.8±147.3	773.9±120.1
Time Spent in Movement (sec)				
13	491.2±340.7	419.6±265.9	443.6±304.2	487.2±424.2
17	817.4±387.1	1012.1±454.9	1046.1±406.9	1110.1±563.6
21	881.3±263.9	750.2±367.9	841.7±399.7	897.6±478.8
60	1742.9±387.1	1755.6±313.1	1824.0±469.7	1733.7±397.1
Females				
Number of Movements (counts)				
13	389.0±167.6	522.4±182.6	502.1±240.7	435.8±176.5
17	583.1±226.5	660.7±167.8	674.9±160.6	643.4±148.8
21	470.6±181.2	544.0±219.3	574.9±181.9	548.4±188.5
60	761.7±151.4	763.2±131.6	821.6±129.1	798.7±109.1
Time Spent in Movement (sec)				
13	535.9±323.3	670.2±342.8	716.0±402.6	591.4±322.2
17	955.0±400.6	1080.9±366.5	1122.3±316.0	1116.3±436.8
21	716.3±318.6	817.1±408.7	955.2±366.7	882.3±381.8
60	1834.7±374.0	1754.3±418.4	1861.1±357.1	1893.7±335.5

a Data were obtained from Table F1, pages 439-446 of the study report; n=16-20.

c. **Auditory startle habituation** - No treatment-related effects on response magnitude or average response magnitude (over 5 blocks) were observed on PNDs 22 or 60 in either sex (Table 10). Habituation was observed over Blocks 1-5 at all doses at both test sessions. Time to peak response was not reported.

Table 10. Mean (±SD) auditory startle habituation magnitude (g) data from F_1 rats in Subset 3. [a]

Observation [b]		Dose (mg/kg/day)			
		0	10	50	200
Males					
PND 22	Block 1	18.67±15.55	19.21±11.51	23.57±14.94	21.18±12.38
	Block 2	11.77±10.03	13.41±8.70	16.03±10.92	16.04±11.32
	Block 3	12.30±9.60	12.45±7.60	13.15±8.91	11.99±8.47
	Block 4	12.41±11.06	12.17±7.28	16.36±11.75	12.33±8.43
	Block 5	12.23±10.69	14.14±8.50	18.51±16.00	14.23±9.85
	Average	13.475±10.758	14.279±7.299	17.520±11.283	15.156±9.291
PND 60	Block 1	94.11±80.98	94.24±68.58	99.11±71.23	82.96±39.79
	Block 2	49.67±42.63	32.41±23.15	58.68±50.58	59.54±26.39
	Block 3	34.93±28.95	36.52±28.44	44.63±36.16	40.84±35.97
	Block 4	32.55±24.70	29.83±23.21	33.34±25.52	44.48±33.53
	Block 5	32.64±31.47	21.42±17.44	33.87±24.25	36.48±20.37
	Average	48.755±36.481	42.889±28.369	53.920±35.686	52.856±26.109
Females					
PND 22	Block 1	19.19±10.10	21.76±12.63	18.79±10.09	20.40±11.70
	Block 2	13.13±8.48	16.76±12.08	12.17±8.95	17.53±11.77
	Block 3	11.55±5.94	17.28±12.36	10.73±6.98	15.38±11.45
	Block 4	12.21±7.68	16.24±14.64	11.59±7.03	13.56±10.72
	Block 5	13.44±11.11	13.61±10.83	13.61±9.86	14.47±10.88
	Average	13.905±7.106	17.120±10.991	13.380±7.448	16.268±9.917
PND 60	Block 1	48.07±21.60	55.82±35.93	52.41±39.18	68.15±53.47
	Block 2	23.01±13.39	33.34±24.22	37.68±30.07	42.97±29.11
	Block 3	19.67±13.19	29.61±24.09	26.07±21.74	33.81±34.11
	Block 4	15.64±13.94	26.17±22.18	22.85±18.67	23.92±25.58
	Block 5	19.11±19.94	24.12±17.46	17.86±14.74	20.71±19.40
	Average	25.090±12.791	33.810±21.620	31.360±21.930	37.911±28.828

a Data were obtained from Table F2, pages 447-448; n=16-20.
b Block=10 consecutive trials

d. Learning and memory testing - No treatment-related differences in learning or memory were noted in any treated group relative to concurrent controls in the passive avoidance or water maze tests (Tables 11 and 12, respectively).

Table 11. Mean (±SD) passive avoidance performance data in F_1 rats in Subset 2 administered Tetrachlorvinphos daily via gavage from PND 7-21. [a]

Session/Parameter		Dose (mg/kg/day)			
		0	10	50	200
Males					
Session 1 PND 22	Trials to criterion	4.9±1.5	4.4±0.9	4.8±1.5	4.0±1.0
	Latency trial 1 (sec)	5.9±4.2	6.2±3.7	6.6±3.1	10.0±12.3
	Latency trial 2 (sec)	33.4±26.2	31.2±19.7	28.7±23.4	36.1±20.3
	Failed to learn (n)[b]	0	0	0	0
Session 2 PND 29	Trials to criterion	3.4±2.2	3.3±1.1	3.3±1.1	2.9±0.9
	Latency trial 1 (sec)	28.8±25.5	28.3±20.7	16.2±18.0	26.2±25.8
Females					
Session 1 PND 22	Trials to criterion	4.3±1.1	4.4±1.2	5.0±1.7	3.7±0.5
	Latency trial 1 (sec)	8.8±4.9	11.2±10.2	9.6±5.5	8.6±6.8
	Latency trial 2 (sec)	29.2±21.6	36.4±24.0	30.0±21.6	37.7±19.2
	Failed to learn (n)[b]	0	0	0	0
Session 2 PND 29	Trials to criterion	3.3±1.0	3.4±0.9	3.8±2.1	4.2±2.9
	Latency trial 1 (sec)	27.7±23.5	19.6±18.8	23.2±21.9	23.6±22.2

a Data were obtained from Table E1, page 420 of the study report; n=19-20.
b The values for rats that did not meet the criterion in Session 1 were excluded from group averages and statistical analyses in Session 2.

Table 12. Mean (±SD) water maze performance data in F_1 rats in Subset 2 administered Tetrachlorvinphos daily via gavage from PND 7-21. [a]

Session/Parameter		Dose (mg/kg/day)			
		0	10	50	200
Males					
Session 1 PND 58	Trials to criterion	9.0±2.9	9.5±3.6	9.3±2.7	10.0±3.1
	Errors per trial	0.54±0.64	0.49±0.25	0.46±0.27	0.58±0.43
	Latency trial 2 (sec)	20.2±17.3	16.9±11.9	17.7±13.3	20.9±16.8
	Failed to learn (n)[b]	2	4	2	2
Session 2 PND 65	Trials to criterion	6.7±3.3	6.3±2.8	6.9±3.4	7.1±3.3
	Errors per trial	0.08±0.14	0.07±0.12	0.10±0.19	0.13±0.17
	Latency trial 1 (sec)	7.6±5.0	6.7±4.4	7.8±7.0	8.9±6.2
Females					
Session 1 PND 58	Trials to criterion	9.3±3.1	7.8±2.2	8.9±3.1	10.6±3.3
	Errors per trial	0.50±0.37	0.47±0.42	0.40±0.15	0.48±0.38
	Latency trial 2 (sec)	17.9±13.3	17.3±17.1	16.2±11.4	13.9±11.9
	Failed to learn (n)[b]	1	0	1	2
Session 2 PND 65	Trials to criterion	6.0±2.4	6.7±3.1	7.0±3.0	8.2±3.7
	Errors per trial	0.07±0.13	0.10±0.15	0.15±0.25	0.21±0.22
	Latency trial 1 (sec)	7.8±4.3	7.6±3.5	8.9±4.8	7.7±3.5

a Data were obtained from Table E2, page 421 of the study report; n=15-20.

b The values for rats that did not meet the criterion in Session 1 were excluded from group averages and statistical analyses in Session 2.

5. Postmortem results

a. Brain weights - No treatment-related differences in absolute or relative (to body) brain weights were noted between treated and control groups on PND 21 in either sex (Table 13). On PND 70, absolute brain weight was decreased ($p \leq 0.01$) by 8% in the 200 mg/kg/day males compared to controls. This finding was judged to correlate with the treatment-related decreases in several microscopic linear brain measurements (striatum, corpus callosum, hippocampus, and cerebellum). Relative brain weights in both sexes and absolute brain weights in the females were similar to controls on PND 70.

Table 13. Mean (±SD) brain weights in F_1 rats administered Tetrachlorvinphos daily via gavage from PND 7-21. [a]

Parameter	Dose (mg/kg/day)			
	0	10	50	200
Males				
PND 21 (subset 1)				
Body Weight (g)	47.4±8.9	50.4±9.6	53.4±6.0	45.9±8.4
Brain Weight (g)	1.68±0.22	1.70±0.14	1.67±0.12	1.56±0.11
Brain-to-body weight ratio (%)	3.591±0.402	3.513±0.822	3.143±0.221	3.489±0.494
PND 70 (subset 4)				
Body Weight (g)	429.1±42.3	411.2±35.2	397.5±42.3	424.4±42.1
Brain Weight (g)	2.31±0.13	2.26±0.10	2.23±0.06	**2.13±0.08** (↓8)**
Brain-to-body weight ratio (%)	0.540±0.052	0.552±0.036	0.567±0.068	0.507±0.044
Females				
PND 21 (subset 1)				
Body Weight (g)	48.7±5.2	45.0±7.1	44.8±8.1	48.4±6.0
Brain Weight (g)	1.69±0.15	1.59±0.12	1.54±0.16	1.58±0.09
Brain-to-body weight ratio (%)	3.489±0.302	3.601±0.480	3.486±0.395	3.280±0.359
PND 70 (subset 4)				
Body Weight (g)	240.1±18.7	244.0±28.9	236.8±16.0	238.5±16.8
Brain Weight (g)	2.01±0.10	2.09±0.09	1.97±0.14	1.92±0.12
Brain-to-body weight ratio (%)	0.843±0.074	0.866±0.101	0.835±0.044	0.807±0.058

a Data were obtained from Tables D1-D2 and G1-G2, pages 413-414 and 530-531; n=8-10 for Subset 1 and 10 for Subset 4. Percent difference from controls (calculated by reviewers) is listed parenthetically.

** Statistically significantly different from controls at $p \leq 0.01$

b) Neuropathology

1) Macroscopic examination - No treatment-related gross lesions were noted at any dose. The majority of the adverse observations occurred in the animals that were found dead or moribund sacrificed as a result of dosing accidents.

2) Microscopic examination - For ease of data evaluation, the reviewers converted the micrometer scale values for morphometric measurements, listed in the pathology report, to µm using the magnification factor listed in the column headers within the data tables from the study report. The reviewers indicated statistical significance in the tables below based on the Text Tables from the study report. No additional statistical evaluations were performed by the reviewers.

No treatment-related histo- or neuropathology findings were noted at any dose. Morphometric brain measurements for the animals sacrificed on PND 21 (Subset 1) and 70 (Subset 4) are summarized below in Tables 14a and 14b, respectively. At 200 mg/kg/day, decreases ($p \leq 0.05$-0.01) in morphometric brain measurements included the following: (i) thickness of the striatum on PND 21 (↓4-5% both sexes) and PND 70 (↓7% males only); (ii) thickness of the corpus callosum on PND 70 (↓16-21% both sexes); (iii) thickness of the hippocampal gyrus on PND 70 (↓7-9% both sexes); and (iv) height of the cerebellum on PND 70 (↓7% males only).

At 50 mg/kg/day, increased thickness of the striatum in the PND 21 males (↑5%; $p \leq 0.05$) was observed. The reviewers agree with the Sponsor that this finding was unrelated to treatment as this was in contrast to the decrease observed in the 200 mg/kg/day dosage group.

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS - The investigators concluded that there was no evidence of maternal toxicity at any dose. Offspring toxicity at 200 mg/kg/day was characterized by decreases in body weight gains during pre-weaning in both sexes and post-weaning in the females, absolute brain weight in the males on PND 70, and several microscopic linear brain measurements in both sexes on PNDs 21 and 70. The maternal NOAEL was 200 mg/kg/day. The offspring NOAEL was 50 mg/kg/day.

B. REVIEWER'S COMMENTS -

In dams, there were no treatment-related effects on mortality, clinical signs, body weight, body weight gain, feed consumption, FOB, or gross pathology. No treatment-related effects on reproductive parameters were observed. **The maternal LOAEL was not observed. The maternal NOAEL was 200 mg/kg/day.**

In offspring, there were no treatment-related effects on viability, litter observations, clinical signs, body weight, food consumption, FOB, motor activity, acoustic startle habituation, learning and memory (passive avoidance and water maze), gross pathology, or histopathology parameters. At 200 mg/kg/day, decreases ($p \leq 0.05$) in body weight were noted in the males (↓5-8%) on PNDs 15 and 16 (pre-weaning) and PND 22 (post-weaning) and in the females (↓6%) on PND 29 (post-weaning). Body weight gains were decreased ($p \leq 0.05$) during pre-weaning at several intervals in the males (↓12-28%) and females (↓13-26%). Decreases ($p \leq 0.05$) in body weight gains during post-weaning were observed in the males on PNDs 21-22 (↓36%) and in the females on PNDs 21-22 and 22-29 (↓7-28%). Absolute brain weight was decreased ($p \leq 0.01$) by 8% in the males. This finding was judged to correlate with the treatment-related decreases ($p \leq 0.05$) in several microscopic linear brain measurements: (i) thickness of the striatum on PND 21 (↓4-5% both sexes) and PND 70 (↓7% males only); (ii) thickness of the corpus callosum on PND 70 (↓16-21% both sexes); (iii) thickness of the hippocampal gyrus on PND 70 (↓7-9% both sexes); and (iv) height of the cerebellum on PND 70 (↓7% males only).

No treatment related effects were noted in the ≤50 mg/kg/day F_1 offspring.

The offspring LOAEL was 200 mg/kg/day, based on decreases in body weight, body weight gains and several morphometric linear brain measurements in both sexes, and decreased absolute brain weight in the males on PND 70. The offspring NOAEL was 50 mg/kg/day.

This study is classified **as Acceptable/Non Guideline** and may be used for regulatory purposes, however it does not satisfy the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, §83-6); OECD 426 at this time pending a comprehensive review of all available positive control data.

C. DISCUSSION - The DNT Work Group also reviewed summary result of the comparative cholinesterase assay (CCA) studies (MRID 46036001) with TCVP, and determined that a complete report including the raw data for the CCA studies is required in order for the Work Group to evaluate the CCA studies since only summaries of the results of these studies were submitted.

D. STUDY DEFICIENCIES - The following deficiencies were noted, but do not alter the conclusions of this DER:

- The dose formulations were only marginally within the acceptable range of percent nominal.
- FOB tabular data were not provided (a major reporting deficiency).

Table 14a. Mean (±SD) morphometric data for F_1 rats on PND 21 (Subset 1). [a]

Parameter	Dose (mg/kg/day)			
	0	10	50	200
Males				
Cerebrum (mm)	14.04±0.36	14.33±0.42	14.17±0.48	13.79±0.40
Cerebellum (mm)	6.43±0.50	6.56±0.32	6.50±0.29	6.31±0.35
Frontal Cortex (μm)[b]	1818.33±102.50	NP	NP	1818.00±103.39
Parietal Cortex (μm)[b]	1840.00±121.40	NP	NP	1839.00±97.78
Striatum (μm)[bc]	2645.63±93.14	NP	**2768.33±91.28* (↑5)**	NA
Striatum (μm)[bc]	2749.33±82.37	NP	NA	**2647.20±69.79* (↓4)**
Corpus Callosum (μm)[b]	178.00±18.97	NP	NP	167.40±16.60
Hippocampal Gyrus (μm)[b]	1163.33±85.55	NP	NP	1110.00±58.74
Cerebellum midline (μm)[b]	4946.67±371.21	NP	NP	4680.00±316.23
Females				
Cerebrum (mm)	13.94±0.35	13.85±0.41	13.77±0.60	13.91±0.25
Cerebellum (mm)	6.54±0.46	6.59±0.31	6.40±0.45	6.21±0.36
Frontal Cortex (μm)[b]	1923.00±106.25	NP	NP	1856.67±66.14
Parietal Cortex (μm)[b]	1890.00±68.56	NP	NP	1866.67±54.66
Striatum (μm)[bc]	2791.67±84.00	NP	2683.50±116.72 (↓4)	NA
Striatum (μm)[bc]	2908.80±127.40	NP	NA	**2757.33±100.00* (↓5)**
Corpus Callosum (μm)[b]	184.80±24.30	NP	NP	165.00±16.97
Hippocampal Gyrus (μm)[b]	1170.00±49.50	NP	NP	1140.00±66.24
Cerebellum midline (μm)[b]	5166.00±271.96	NP	NP	4986.67±151.33

a Data were obtained from Text Tables 5-6, page 69; n=9-10. Percent difference from controls (calculated by reviewers) is listed parenthetically.

b Calculated by the reviewers, as a mean of left and right values, using micrometer gauge measurements and magnification conversion factors from Text Tables 9-12, pages 72-75.

c The control and 50 mg/kg/day groups were evaluated at a magnification of 30x. Because the 200 mg/kg/day group was evaluated at 48x, the controls were re-evaluated at 48x for comparison.

* Statistically significantly different from controls at $p \leq 0.05$ as indicated in the raw data contained within Text Tables 5 or 6.

NP Not performed

NA Not applicable

Table 14b. Mean (±SD) morphometric data for F_1 rats on PND 70 (Subset 4). [a]

Parameter	Dose (mg/kg/day)			
	0	10	50	200
Males				
Cerebrum (mm)	15.28±0.44	15.36±0.66	15.33±0.35	15.02±0.37
Cerebellum (mm)	6.72±0.38	6.63±0.25	6.68±0.32	6.63±0.36
Frontal Cortex (µm)[b]	1950.00±75.17	NP	2025.00±69.28	1909.5±79.39
Parietal Cortex (µm)[b]	1984.50±76.17	NP	2044.50±85.45	1957.50±73.57
Striatum (µm)[b]	3206.40±100.69	NP	3240.00±131.45	**2992.80±84.70** (↓7)**
Corpus Callosum (µm)[b]	293.40±24.40	NP	271.20±23.46	**245.40±27.49** (↓16)**
Hippocampal Gyrus (µm)[b]	1446.00±64.11	NP	1420.50±38.11	**1318.50±56.32** (↓9)**
Cerebellum midline (µm)[b]	5448.00±247.87	NP	5514.00±293.19	**5040.00±257.68** (↓7)**
Females				
Cerebrum (mm)	14.83±0.40	15.02±0.58	14.59±0.39	14.63±0.26
Cerebellum (mm)	6.52±0.27	6.61±0.36	6.37±0.42	6.41±0.39
Frontal Cortex (µm)[b]	1855.00±85.84	NP	1857.00±73.42	1869.00±75.56
Parietal Cortex (µm)[b]	1880.00±100.90	NP	1903.50±73.98	1893.00±84.50
Striatum (µm)[b]	2937.0±170.53	NP	3040.80±83.18	2971.20±112.46
Corpus Callosum (µm)[b]	268.80±33.08	NP	234.00±28.30 (↓13)	**212.40±27.60** (↓21)**
Hippocampal Gyrus (µm)[b]	1392.00±67.38	NP	1323.00±51.87 (↓5)	**1299.00±78.80* (↓7)**
Cerebellum midline (µm)[b]	5088.00±290.93	NP	5028.00±296.38	4980.00±243.31

a Data were obtained from Text Tables 7-8, page 70; n=10. Percent difference from controls (calculated by reviewers) is listed parenthetically.

b Calculated by the reviewers using micrometer gauge measurements and magnification conversion factors from Text Tables 13-16, pages 76-79.

* Statistically significantly different from controls at $p \leq 0.05$ as indicated in the raw data contained within Text Tables 7 or 8.

** Statistically significantly different from controls at $p \leq 0.01$ as indicated in the raw data contained within Text Tables 7 or 8.

NP Not performed

NA Not applicable

DATA FOR ENTRY INTO ISIS

Developmental Neurotoxicity Study - rats (870.6300)

PC code	MRID #	Study type	Species	Duration	Route	Dosing method	Dose range mg/kg/day	Doses tested mg/kg/day	NOAEL mg/kg/day	LOAEL mg/kg/day	Endpoint(s)	Comments
083701	46660601	dev neurotox	rats	GD 6-LD 6 PND 7-21	oral	gavage	10-200	0, 10, 50, 200	200	Not observed		Maternal
083701	46660601	dev neurotox	rats	GD 6-LD 6 PND 7-21	oral	gavage	10-200	0, 10, 50, 200	50	200	Decr BWG, brain weight (males), morphometric brain measurements	Offspring

13544

R158366

Chemical: Tetrachlorvinphos

PC Code: 083701
HED File Code: 13000 Tox Reviews
Memo Date: 3/19/2008
File ID: TX0053833
Accession #: 000-00-0125

HED Records Reference Center
5/8/2008